

Neutral Fragment Filter

PSB 103

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A new feature in Xcalibur™ 1.2 lets you apply a neutral fragment filter to MS² data files. This will allow post-acquisition data mining for specific neutral losses of product ion MS² files acquired on a trap.

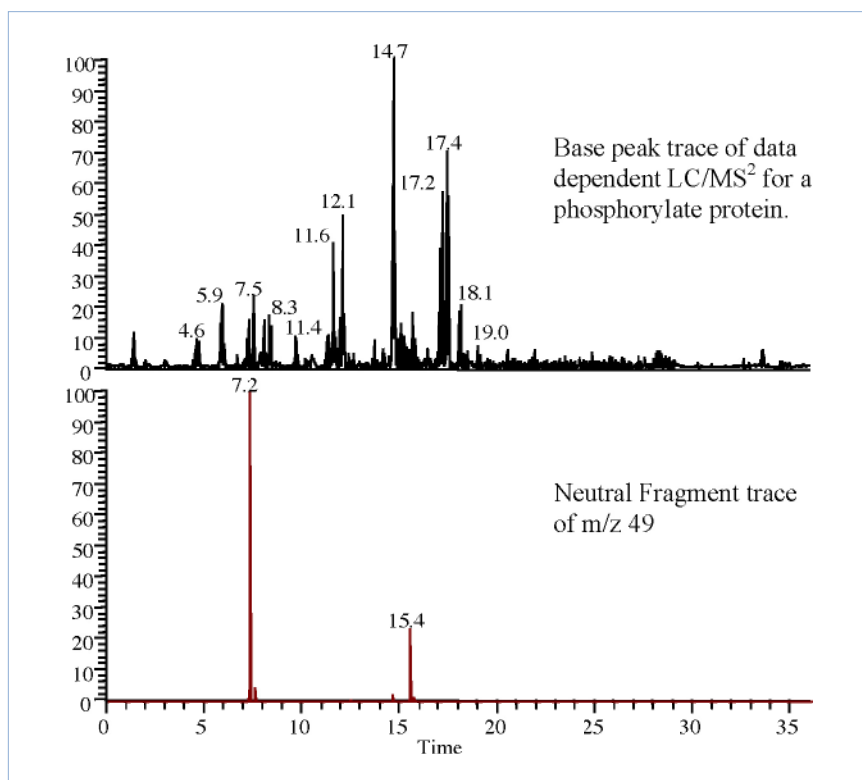


Figure 1. Base peak and neutral fragment trace for a tryptic digest of three proteins.

Data Dependent™ MS² conditions. The lower trace shows the neutral loss trace of m/z 49, which suggests the presence of two major phosphorylation sites.

An example of this technique is the search for post-translational modifications, such as phosphorylations in complex protein digests. Under product ion MS² conditions, peptides that are phosphorylated at serine or threonine lose m/z 98 and m/z 49 (HPO₃) from singly- and doubly-charged parent ions respectively. It is therefore relatively easy to find these MS² neutrals if the correct filter is applied.

The new neutral fragment filter steps through each MS² experiment looking for the specific neutral loss. It plots the presence of an ion at that loss from the parent. Figure 1 shows the base peak trace from a mixture of three digested proteins under

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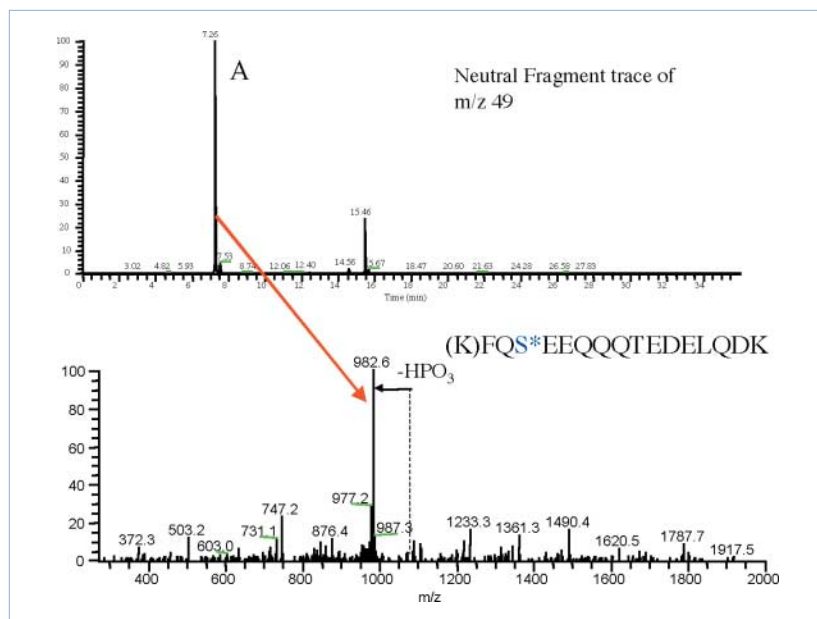


Figure 2. Neutral fragment trace and MS² spectrum from phosphorylated peptide A.

Figure 2 shows the spectrum associated with peak A which has a characteristic base ion at m/z 49 from the parent, and is indeed phosphorylated.

This type of filter could be used for any experiment such as a natural product or metabolite screen. Figure 3 shows a complex profile of metabolites from the microsomal incubation of the drug buspirone. A characteristic MS² loss for this particular sample is the loss of m/z 164, indicating the presence of metabolites with a pyrimidinyl piperazine structure. By applying the neutral loss filter to this trace, a group of ions were extracted that represent the target metabolites.

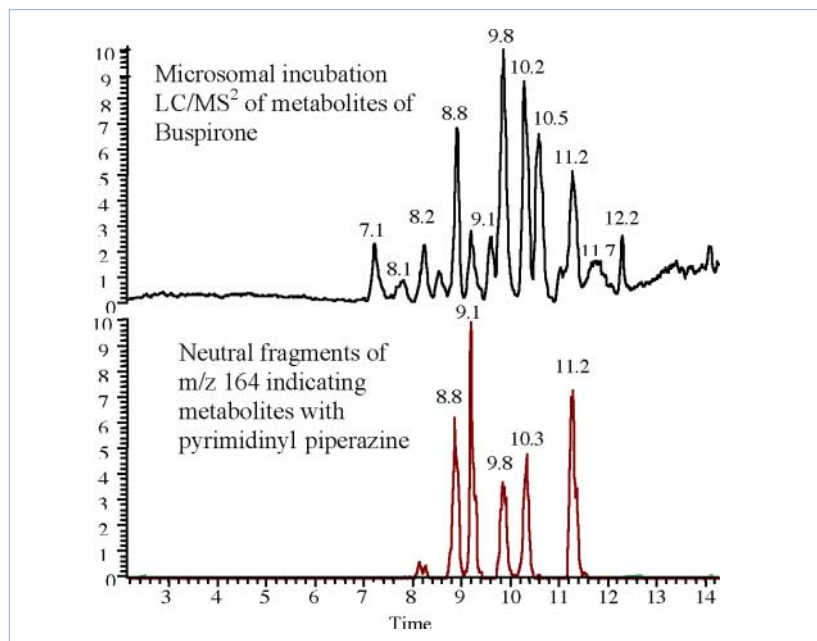


Figure 3. Metabolite LC/MS² screen with applied neutral fragment trace.